

**STANDARD TOTAL COLIFORM FERMENTATION TECHNIQUE****SM 18<sup>th</sup>/19<sup>th</sup>/ 20<sup>th</sup> 9221 B****Page 1 of 3**

Facility Name: \_\_\_\_\_ VELAP ID \_\_\_\_\_

Assessor Name: \_\_\_\_\_ Analyst Name: \_\_\_\_\_ Inspection Date \_\_\_\_\_

**Relevant Aspect of Standards****Method  
Reference****Y****N****N/A****Comments***Records Examined:* SOP Number/ Revision/ Date \_\_\_\_\_ Analyst: \_\_\_\_\_

Sample ID: \_\_\_\_\_ Date of Sample Preparation: \_\_\_\_\_ Date of Analysis: \_\_\_\_\_

When drinking waters were analyzed, were completed tests in 9221B.3 or either the positive EC broth in 9221E or the positive EC MUG broth in 9221F applied to not less than 10% of all coliform-positive samples per quarter?

9221 A. Intro  
1

When nonpotable waters were analyzed, was the completed test procedure 9221B.3 used on at least 10% of coliform-positive samples on a seasonal basis?

9221 A. Intro  
2**Presumptive Phase**

Was lauryl tryptose broth used?

9221 B 1

If medium was refrigerated after sterilization, was it incubated at room temperature overnight prior to use so that tubes showing growth and/or bubbles could be discarded?

9221 B 1

When inverted gas vials were omitted, was 0.01 g/L of bromocresol purple added to determine acid production?

9221 B 1 a 1

Was lauryl triptose broth prepared using Table 9221:I as a guide?

9221 B 1 a 1

Were samples and dilutions shaken vigorously 25 times prior to inoculations?

9221 B 1 b 1

Were inoculated tubes or bottles incubated at  $35 \pm 0.5^{\circ}\text{C}$  and examined for growth, gas, and acidic reaction after  $24 \pm 2$  hours?

9221 B 1 b 2

Notes/ Comments:

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Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
If no gas or acidic reactions were evident after 24 ± 2 hours, were inoculated containers reincubated and reexamined after 48 ± 3 hours?	9221 B 1 b 2				
If inner vial was omitted, was growth with acidity used to signify positive presumptive reactions?	9221 B 1 b 2				
Was gas or acidic reaction within 48 ± 3 hours used to signify positive presumptive reactions?	9221 B 1 c				
<b>Confirmed Phase</b>					
Was brilliant green lactose bile broth used as the fermentation medium?	9221 B 2 a				
Were all presumptive tubes showing growth, gas, or acidic reaction within 24 ± 2 or 48 ± 3 hours submitted to the confirmed phase? (For waters or wastewaters known to be consistently polluted only the highest dilution need be moved to confirmed.)	9221 B 2 b				
Were positive presumptive tubes shaken or rotated to resuspend the organisms prior to inoculation?	9221 B 2 b				
Were inoculated brilliant green tubes incubated at 35 ± 0.5°C?	9221 B 2 b				
Was gas formation at any time within 48 ± 3 hours used to indicate positive confirmed phase?	9221 B 2 b				
Were MPNs calculated using 9221C?	9221 B 2 b				
<b>Completed Phase (NOT APPLICABLE if using EC and EC MUG)</b>					
Was LES Endo agar refrigerated in the dark and kept not longer than 3 weeks (SM 19 <sup>th</sup> ) or 2 weeks (SM 20 <sup>th</sup> )?	9222 B 2 a				
Were nutrient agar tubes solidified so the agar surfaces were slanted?	9221 B 3 a 3				
Was one LES Endo or MacConkey agar plate streaked from each brilliant green tube showing gas?	9221 B 3 b 1				
Were inoculating needles or loops sterilized prior to streaking, and was care taken to avoid scratching agar surfaces during streaking?	9221 B 3 b 1				
Were plates inverted and incubated at 35 ± 0.5°C for 24 ± 2 hours?	9221 B 3 b 1				
Notes/Comments:					

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Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
Were "typical" positive colonies transferred into lauryl tryptose fermentation tube and onto a nutrient agar slant? (Agar slants not required for drinking water.) <i>(Typical colonies for LES Endo are pink to dark red with green metallic sheen; for MacConkey they are red and may be surrounded by an opaque zone of precipitated bile.)</i>	9221 B 3 b 2				
Were secondary lauryl tryptose tubes incubated at 35 ± 0.5°C for 24 ± 2 hours and examined for gas?	9221 B 3 b 2				
If no gas was found in lauryl tryptose tubes after 24 hours, were they incubated again for 48 ± 3 hours, and reexamined for gas?	9221 B 3 b 2				
Was the gram-stain technique used on those 24-hour nutrient agar slants corresponding to the secondary tubes that showed gas? (Not including drinking water.)	9221 B 3 b 2				
Did examinations of gram-stained preparations showing gram-negative (red), non-spore forming, and rod-shaped bacteria constitute a positive result for the completed test?	9221 B 3 c				
18 <sup>th</sup> Ed Only: If no gas was produced in the secondary tube of lauryl tryptose, was the original MPN from the confirmed test adjusted accordingly?	9921 B 3 c				
Notes/Comments:					